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ON THE NEURONAL BASIS OF COGNITION: CELL-TYPE SPECIFIC CIRCUITRY AND FUNCTIONS OF THE PREFRONTAL CORTEX

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On the Neuronal Basis of Cognition: Cell-type Specific Circuitry and Functions of the Prefrontal Cortex

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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ABSTRACT

This thesis recapitulates the history of research, and current knowledge, of the prefrontal cortex (PFC) in order to provide a context for the included scientific articles. The evident, but ill-defined, symptoms of a perturbation of the PFC is still a conundrum to neuroscientists. Though considered a source of cognition, or intellect, the quest to define an overall framework of how cognition is processed, or built, in the PFC, is very much an ongoing endower. The work presented in this thesis address both the structural architecture, as well as the electrophysiological properties, underlying the unique functions of the PFC.

Chapter 2 of this thesis discusses the unique connectivity of the PFC and its relevance to a functional understanding of the neuronal computations present in the PFC. In this context, PAPER I reports the local and whole-brain connectivity scheme of discrete neuronal types within the PFC by the use of a novel rabies virus tracing system. Through carefully mapping monosynaptic inputs to four separate neuronal types, we describe that all connectivity traits defining the PFC, hold true for multiple neuronal types: the appearance of subnetworks within the PFC, the distinct thalamic innervation, and the high interconnectivity between PFC subregions.

The third Chapter describes various electrophysiological properties present in the PFC, and more specifically, the occurrence of gamma oscillations, and their specific relevance to cognition. PAPER II and III report on the relevance of parvalbumin expressing interneurons for the generation of gamma oscillations in the rodent cortex. PAPER III further describes the presence of gamma oscillations during correct allocation of attention, and the frequency dependent activity of parvalbumin expressing interneurons. The temporal organisation of parvalbumin expressing interneurons, and the functional activity of excitatory neurons are, at this stage, only observations. However, the activity of parvalbumin expressing neurons was shown to be vital for the attentive state, and consequently, crucial for the network activity of the PFC.

In summary, the work of this thesis, portrays cell type specific activity, as well as local and long-range circuitry of the rodent PFC. Although the concept of cognition may differ in appearance in mice as compared to humans, there is a common acceptance that key elements in the structure and function of the brain have been conserved through evolution, allowing for translatability. Ultimately, by carefully disentangling and observing small pieces of the circuitry and neuronal computation at a time, we can begin to build a framework for the neuronal underpinnings of cognition.

LIST OF SCIENTIFIC PAPERS

I. A whole brain atlas of the monosynaptic input targeting four different cell-types in the Prefrontal cortex of the mouse.
Nature Neuroscience. 2019 Apr;22(4):657-668.
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II. Genetic targeting and manipulation of parvalbumin neurons in the rat.
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Brunner H, **Ährlund-Richter S**, van Luteren JA, Kim H, Crestani AP, Meletis K, Carlén M.

III. Prefrontal Parvalbumin Neurons in Control of Attention.
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Berndt A, Lee SY, Wietek J, Ramakrishnan C, Steinberg EE, Rashid AJ, Kim H, Park S, Santoro A, Frankland PW, Iyer SM, Pak S, **Ährlund-Richter S**, Delp SL, Malenka RC, Josselyn SA, Carlén M, Hegemann P, Deisseroth K.

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1 THE PREFRONTAL CORTEX, CHAPTER 1

The brain region known as the prefrontal cortex (PFC) is considered indispensable for higher brain functions such as working memory, attention, and decision-making, and many mental disorders are thought to occur due to an imbalance of neuronal activity within the PFC[1]. It is not only the most advanced and evolutionally developed brain region we possess, but also the most sensitive and enigmatic one. This thesis recapitulates the history of research, and current knowledge, of the PFC to provide a context for included scientific articles.

1.1 A historical discovery and understanding of the prefrontal cortex

The first documented use of the term "Prefrontal" is attributed to Ferrier and Yeo in a publication from 1884. In one of the experiments, the authors surgically removed the very front of the brain of a small baboon and after recovery from the surgery observed his behaviour; "*Watched from day to day, it exhibited no defects as regards to any of its movements, ocular or otherwise, or as regards any of its sensory faculties, which where tested in various ways. Only its manner seemed changed, and this was noted by all who had seen its former vivacity. It lost all its fun and trickiness, seemed not to know its name, took little or no interest in its companions, and was very easily cowed by them."* [2]. The work by Ferrier and Yeo was not primarily dedicated to describing or defining the function of the region they named the PFC. Although, the authors summarised the effect of the lesion, and perhaps the function of the PFC as well as any neuroscientist today; as very evident but difficult to define.

At this point in history, the first clinical case study of a PFC lesion had already occurred, in what perhaps is the most famous accident in cognitive neuroscience. The railroad worker Phineas Gage's skull was penetrated by a metal rod in 1849, thereby creating a lesion of his frontal lobes, the region later referred to by Ferrier as the PFC. Gage miraculously survived, and although the most common told story of today is of a man who's personality was significantly altered by the accident, the condition of Gage's intellect and personality after the accident was widely debated at the time[3]. Gage's symptoms where described very differently by two separate physicians and the debate was centred on the presence (or lack) of 'mental manifestations' after the accident.

The vigorous dispute regarding the cognitive change of Phineas Gage during the 19th century was fuelled by the simultaneous debate regarding the theory of *cerebral localization*, the concept of cognitive or sensory functions being attributed to a precise location within the brain[3]. The presence of metal manifestations could therefore serve as evidence of the importance of the frontal lobes for a functional intellect. Conversely, a lack of manifestation could confirm the notion that no single region carried the responsibility of

a specific function. Thirty years after Gage's accident, David Ferrier, who would be the first to coin the term 'Prefrontal', used the case study of Phineas Gage as a keystone for his modern theory of the functions of the frontal lobes, and this is how we remember Phineas Gage today[3,4]. The theory of cerebral localization became established, and the PFC, the location of the human intellect.

The aim to describe unique regions of the brain continued and culminated in 1909 with the work of Korbinian Brodmann, and his 52 areas of the cortex[5]. Broadmann's 52 areas are based on cytoarchitectural patterns, not functions, of the human and monkey neocortex. The prefrontal cortex, referred to as the 'frontal granular cortex' by Broadmann, was a unique structure, with a distinct granular layer 4, only found in humans and primates and not in any other mammals. Due to the lack of a layer 4 in the frontal lobes of non-primate mammals, the PFC was a structure thought to be unique to higher order mammals and simply not present in other animals.

These three pieces of history; Ferrier's baboon, the case study of Phineas Gage and Brodmann's cortical areas are still relevant to neuroscience today. Although more than a century has passed, key questions about the PFC remain. The very obvious, but ill-defined symptoms of a perturbation of the PFC is still a conundrum to neuroscientists, and the quest to define the overall function of the PFC is very much an ongoing endower. The physical boundary and appearance of the PFC can still today be difficult to determine. Moreover, in the spirit of 'cerebral localizationists', a functional specialization of the subregions within the PFC is even more challenging to define. While there is a common acceptance of the presence of a PFC in other mammals, the level translatability to humans can still be a source of debate, and for a long time primates were the main species in which the function of the PFC was investigated.

1.2 Setting the stage in the primate cortex

Originally 'discovered' and anatomically defined in primates, the experimental investigations underlying contemporary theories of the PFC function also stem from primate work[1,6,7]. The historical lesion study of primates described above, portrayed a behavioural change that was evident but difficult to describe, after a surgical removal of the PFC[2]. In modern day primate work, neuronal activity or discrete lesions of the PFC are correlated to behavioural events in more and more refined behavioural tasks. The PFC is still considered a source of cognition (e.g. the mental process of acquiring knowledge about the world and utilizing it), and numerous functions have been ascribed to it[1]. Within these functions there are prominent theoretical frameworks of how cognition is processed in the primate brain; PFC subregions' functional specificity[8], rule based learning[9] and executive control or 'top-down control'[6] (among others).

The theory of “top-down control” is centred on the neuronal mechanisms behind the communication of the PFC and other brain regions. At large, the PFC is suggested to guide, or bias, the activity in other brain regions to generate an appropriate goal-directed behaviour. A goal-directed behaviour can be driven by an internal goal (e.g. hunger), and one fundamental role of the PFC is to formulate complex plans to achieve the internal goal (e.g. find food). The PFC would then influence the activity in down-stream regions to efficiently gather information relevant to the goal and generate an appropriate behavioural response, or output[6,10–13].

A common experimental framework used to study the importance of the PFC in goal-directed behaviour is visual attention. Recordings of electrophysiological activity in the primate brain can thereafter be correlated to a correct allocation of visual attention[14–18]. One profound discovery in the primate, among others, is the synchrony of neuronal activity between the PFC and primary visual areas during correct allocation of attention[15,19] (*see On the neuronal basis of cognition*).

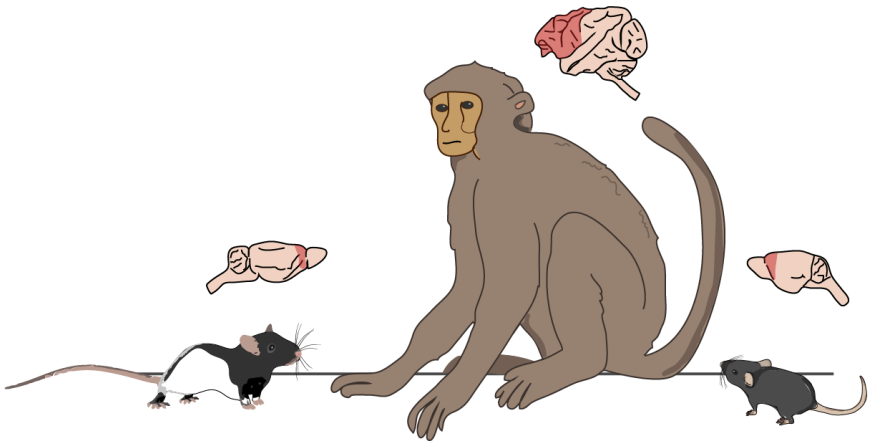

Figure 1. The body and brain of a rat (*Rattus norvegicus*), rhesus macaque (Macaca mulatta) and mouse (Mus musculus). The PFC of each species is marked in red.

1.3 The Prefrontal cortex of rodents

As mentioned previously, the early definition of the PFC by Brodmann indicated that only humans and primates possessed one. This definition of the PFC, to contain a distinct granular layer 4, was challenged by Rose and Woolsey in 1948, when they described the projection field of the mediodorsal nuclei of the thalamus (MD) as a common feature for the PFC across all mammalian species[20]. It is, as of today, the only description of a homological property of the PFC across species. The MD projection to the PFC was confirmed and carefully described in 1968, in the rat, and later in 1981 in the mouse[21,22]. Rose and Woolsey’s definition and the description of the pathway in rodents has not settled the debate regarding the PFC in species other than humans and primates, or the translatability of it [23–25]. It did however provide a common trait for an otherwise enigmatic

brain region with, at the time, ill-defined functions and allowed for experimental investigations of the PFC in the rodent.

Rats (*Rattus norvegicus*) have become invaluable experimental models for neuroscientific research aiming to elucidate the function of the PFC. The cognitive abilities of rats have been repeatedly demonstrated in advanced behavioral tasks investigating working memory[26–29], decision making[30–32] and (visual) attention[26,33,34]. Lesions studies in the rat have clearly linked the necessity of the PFC for goal-directed learning[35], attentional set-shifting[36], decision-making[37] and working memory[38]. Additionally, electrophysiological recordings of neuronal activity in the rat have unfolded neuronal substrates of cognition in the PFC[28–30,34,39–41].

Therefore, the rat has proven to be an instrumental asset in determining the neuronal underpinnings of cognition and the contribution of the PFC in this process. Rats are useful experimental subjects for a wide range of behavioral and electrophysiological investigations in the field of neuroscience and numerous behaviorally complex paradigms are established for this species. However, limited genetic access to subpopulations of neurons in the rat has, (until now) restricted the use of modern neuroscientific techniques.

The use of transgenic mice, and therefore the mouse as a model organism, has become greatly preferable due to the expansion of new techniques allowing for manipulations and recordings of cell-type specific activity[42,43]. The use of the mouse as a rodent animal model in biomedical research has increased from around 20% in the late 1970's to around 50% in more recent years[44]. Arguably, we are approaching a point in time, where the mouse is the species in which we have gathered the most collective knowledge regarding the structure and function of the brain. Consequently, a large body of work in this thesis is done with the use of transgenic mice and forthcoming chapters will predominantly involve work done in mice.

"In investigating the reports on diseases and injuries of the brain I am constantly being amazed at the inexactitude and distortion to which they are subjected by men who have some pet theory to support. The facts suffer so frightfully that I feel obliged always to go to the fountain-head—dirty and muddy though this frequently turns out."

David Ferrier 1877

2 THE PREFRONTAL CORTEX OF THE MOUSE, CHAPTER 2

2.1 The building blocks of mouse PFC

Although the concept of cognition may differ in appearance in mice as compared to humans, there is a common acceptance that key elements in the structure and function of the brain have been conserved through evolution, allowing for translatability. The presence of a conserved functional homology between species, e.g. a brain region in each species dedicated to the same function, is difficult to determine. A structural homology, e.g. gene expression in neurons, is however easier to determine. Therefore, present-day definitions of neuronal types, e.g. defined by RNA/protein expression, are distinct units that can be compared across species. Neuronal types have recently been highlighted as conserved units in amniote brains (mammals, birds and non-avian reptiles), independent of the apparent structural differences between the brains of the different species[45,46]. Consequently, investigations of cell-type specific activity and circuitry are of high importance and may more rapidly reveal functional homologies between species.

2.1.1 *Neuronal types*

The release of either excitatory or inhibitory neurotransmitters (glutamate or GABA, respectively) separate the main populations of neurons in the cortex of the mouse. The large majority of neurons in the PFC of the mouse are excitatory (82%), followed by a lesser fraction of inhibitory neurons (17%) and a small population of neuromodulatory neurons (1%)[47]. Intriguingly, the genetic profiles of glutamatergic or GABAergic neurons differ in two distinct ways. Excitatory, glutamatergic neurons display genetic variability (i.e. RNA-expression) dependent on cortical layer and region. Inhibitory neurons however, display a large variability in genetic profiles, but these are independent from where in the cortex they are situated[48]. Though tremendous effort has been made to classify neuronal types in the mouse brain, we still stand without a single definition of the cell types present in the cortex, we do however have considerable knowledge of what RNA and protein (‘markers’) are present in neurons. Three common molecular markers (proteins) for distinct inhibitory neuronal populations are somatostatin (SST), parvalbumin (PV) and vasointerstinal peptide (VIP). The expression of one of these markers does not indicate an absolute neuronal type, but it does imply the presence of common traits within the local circuit of the neuronal population. Depending on the scientific question asked, or traits investigated, the level of subdivisions between neuronal types mentioned can be more or less significant.

Common properties of PV expressing inhibitory interneurons are their fast firing-rates and high interconnectivity via gap junctions. PV neurons deliver strong direct inhibition via

synapses connecting onto the soma or the axon initial-segment of excitatory neurons[49]. SST expressing interneurons have weaker inhibitory traits, synapsing onto the dendrites of excitatory neurons[50]. VIP expressing neurons are mostly situated in the superficial layers of the cortex, known for their disinhibitory effect on the network, due to their inhibitory synapses onto other GABAergic neurons[51]. PV, SST and VIP expressing cortical interneurons can all be subdivided into additional neuronal populations. They can be further separated by other traits such as morphology or electrophysiological properties, but still sharing the expression of PV, SST or VIP (further subdivisions of PV, SST and VIP interneurons is out of the scope of this thesis, but for review see[52]).

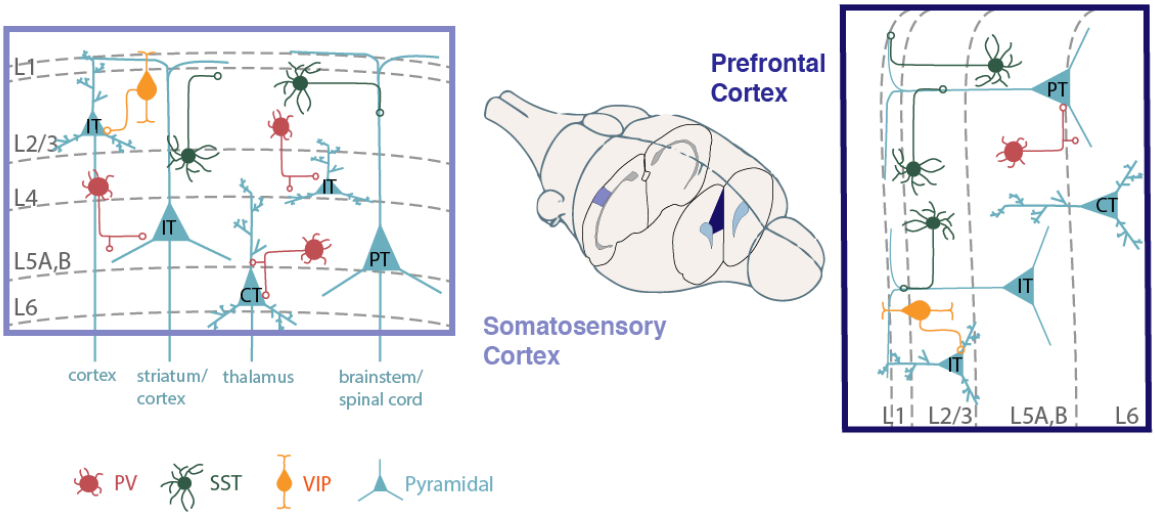

Figure 2. Schematic illustration depicting the layering and distribution of five excitatory and three inhibitory neuronal types in the somatosensory cortex (light purple) and PFC (dark blue) of the mouse. The PFC only holds four layers and a higher abundance of SST interneurons.

2.1.2 *Layers and microcircuitry*

The mouse cortex is suggested to contain a canonical circuit structure, in which the major laminar organization and connectivity patterns are repeated[53] (**Figure 2**). Simplified, the canonical circuit motif is based on the architectural organization of the cortex into layers, and excitatory neurons within each layer belong to one of three main classes distinguished by their gross projection target (intratelencephalic; IT, pyramidal tract; PT or corticothalamic; CT), and the layer in which they harbour their dendrites. Within each class of excitatory neurons (defined by their vertical connectivity), there is a common organization for how the lateral connections stand between neurons in different classes[54]. The interneuron types discussed above can also take part in this canonical circuit, when generalizing their distribution and local connectivity, a homologous pattern can be observed throughout the cortex[54]. It is however important to note that although general principles can be found, a complete circuit has not yet been described for any cortical region[55], and large-scale studies investigating whole-brain connectivity are repeatedly finding connectivity patterns outside the hypothesized scheme[56].

Nevertheless, mapping a canonical circuit, i.e. describing common organization principles for the whole cortex, can be seen as a quest to generate a common 'syntax' for the cortex. By generating a common structural principle, one could potentially discover a common computation, or 'language', spoken throughout the cortex. In parallel, cortical regions are highly functionally specialized, so what underlying factor makes a canonical circuit specialized?

2.1.3 *Brain regions, defining traits of the PFC*

Solemnly observing the neuronal composition of the PFC, in comparison to other cortical regions in the mouse, reveals subtle differences. A recent study compared the composition of interneuron types across all of the cortex of the mouse[57]. Somatomotor cortical regions display a majority of PV interneurons, and sensory regions display a larger VIP population than average in the cortex. Intriguingly, the interneuron composition differed quite profoundly in the PFC in comparison to primary sensory and somatomotor areas, as the subregions of the PFC have the highest proportion of SST expressing interneurons in the mouse cortex[57] (**Figure 2**). SST neurons have been shown to be involved in the formation of tuned neuronal assemblies in the hippocampus[58]. Computational models corroborate the idea of SST neurons regulating incoming input[59], and as the PFC displays task-dependent neuronal assemblies[60] a high level of SST neurons could work in the favour of the PFC to integrate incoming information.

Additionally, and as mentioned earlier, the PFC in the mouse lacks layer 4. The canonical circuit of the cortex described, is foremost based on sensory areas, all possessing 6 layers (L1, 2/3, 4, 5A, 5B and 6)[53,54]. The absence of a granular layer 4 in the PFC of the mouse is however rarely seen as a defining trait of the unique computations present in the PFC, but can instead, to some, be a concern regarding the translatability of the mouse PFC to the human PFC[25].

The most distinct feature of a canonical circuit is understandably not necessarily the neuronal composition. One can rather argue that the information received (and therefore processed) could define separate cortical regions, to the extent that a rewiring of the inputs changes the functionality of a cortical area[61,62]. For the cortex at large, two main connectivity traits can be seen as defining attributes of a cortical region; the input received from the thalamus, and the connectivity to other cortical regions. Each cortical region (as described, so far, by the Allen Reference Atlas[63]) receives unique input from a combination of thalamic nuclei[64]. Secondly, cortical regions that are dedicated to similar functional aspects (and are commonly receiving similar thalamic input) share a higher connectivity to each other, rather than with the rest of the cortex[56]. Groups of cortical regions, when defined by the level of interconnectivity they display, are referred to as modules, or clicks, and these are present in both mice and primates[56,65,66]. The PFC holds these two connectivity traits, i.e. defining it as its own region, as well-defined as any other sensory area of the cortex. More specifically, the PFC receives the distinct innervation of the

mediodorsal thalamus[22] (and additional thalamic nuclei) and the profound interconnectivity within its subregions (grouping it as a module)[56].

2.1.4 *A unique connectivity of the PFC*

Neuronal composition, thalamic innervation and intercortical connectivity are all traits that can add to the differentiation of the PFC from other cortical regions. There are however additional patterns of connectivity that can further deepen our understanding of the local computations and functional properties of the PFC.

Firstly, three distinct connectivity patterns have been identified within the PFC, referred to as subnetworks[64,67–70]. The dorsomedial (dm), ventromedial (vm) and ventrolateral (vl) subnetworks are defined by their efferent and afferent connectivity to the cortex, thalamus, striatum, and other subcortical regions[64,69,70] and each of the PFC subregions group within one of the above subnetworks. The secondary motor cortex (MOs) and the anterior cingulate area (ACA) create the dmPFC subnetwork, the vmPFC subnetwork consists of the prelimbic (PL) and infralimbic area (ILA), and the ventrolateral orbital (ORBvl) and lateral orbital (ORBl) area form the vlPFC subnetwork[67,68]. Combined, the three subnetworks span across the entire cortex, the majority of thalamic nuclei, and subcortical regions such as amygdala, hippocampus, hypothalamus and neuromodulatory systems[64,67–69,71] (First panel, **Figure 3**).

Secondly, the efferent and afferent connectivity of the PFC indicate a highly modulatory effect on other brain regions[56]. Simplified, the cortical layering of projection neuron cell bodies and the downstream axons terminals have been hypothesized to indicate a functional hierarchy between cortical regions in both primate and mouse[72–75]. Projection patterns (i.e. the laminar relationship of the soma and axons of a projection) have mainly been categorized as feedforward or feedback and a cortical region providing modulatory, or feedback, projections to another area will be ranked higher in the hierarchical order[56,72,76]. In short, an area ranked high in an anatomical hierarchical order is suggested to have a modulatory effect on the activity in a lower order area. Conversely, a lower order area provides feedforward input, more commonly seen as a ‘basic’ information transfer.

Recently, the laminar relationship of the connectivity between all cortical regions in the mouse was mapped[56]. The subregions of the PFC, as well as the PFC as a module, displayed a positive hierarchical index, thereby indicating that a majority of the projections stemming from the PFC display anatomical indications of a functional modulatory effect on the downstream cortical target region. Intriguingly, within the PFC module there was no indication of an internal hierarchy, i.e. the projection patterns between PFC subregions display no clear hierarchical organization[56].

To summarize, three distinct connectivity profiles can be found within the subregions of the PFC. However, the PFC subregions are highly interconnected (creating their own cortical module) without any evident internal hierarchy, making the PFC as a whole highly

connected to the whole brain and ideal for integrating brain-wide activity. The PFC displays a pattern of connectivity towards the cortex, indicating a modulatory effect on downstream regions potentially providing evidence for the previously hypothesized model of 'top-down control'[6] (*see On the neuronal basis of cognition*).

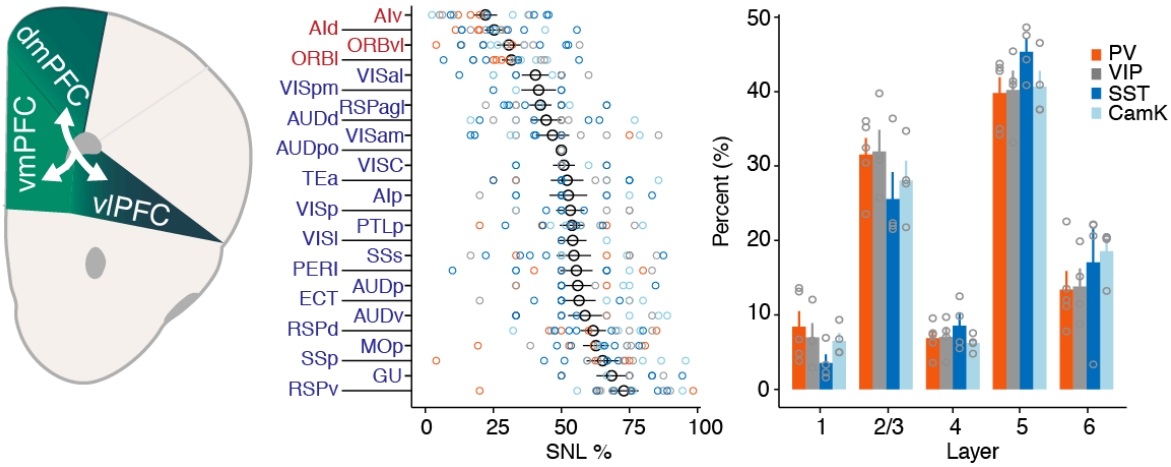

Figure 3. Unique traits of the PFC connectivity. Schematic illustration of the three PFC subnetworks (portrayed in different shades of green). Calculated SLN value (percentage of supragranular labeled neurons) of all cortical input regions to the mPFC, PFC input regions to the mPFC, marked in red, display low SLN values indicating a 'feedback-like' input. Conversely, primary sensory areas, with high SLN, suggest 'feedforward' projections to the PFC. Panel to the right display percentage of input from each cortical layer, to each traced neuronal population. No cortical layer preferentially innervates a discrete neuronal type within the PFC.

2.2 PAPER I

2.2.1 *Purpose and Aim*

As discussed in the earlier sections, neuronal types are suggested to be conserved units across evolution, and still be present in a variety of species[46]. Additionally, the neuronal composition and connectivity profile of the PFC is distinct to other cortical regions, and likely to be highly functionally relevant to the computations executed. Therefore, the investigation of the circuitry and connectivity of distinct neuronal types within the PFC may provide insights to the information the PFC processes within its local circuitry in mice, and may potentially present a framework also relevant for other species.

The Aim of PAPER I was to create a whole-brain map of local and long-range monosynaptic inputs to four distinct neuronal types of the PFC, in order to further elucidate the structural architecture behind the unique functions of the PFC.

2.2.2 *Methodological considerations*

The main methodology applied in PAPER I, was a monosynaptic (glycoprotein deleted) rabies virus system[77]. This method allows for fluorescent mapping of the presynaptic partners of a genetically restricted starter cell (primary infected) population. The starter cell population is restricted due to the cre-dependent expression of an avian-receptor (TVA) (allowing the entry of the pseudotyped rabies virus) and the expression of rabies glycoprotein (enabling the transsynaptic spread)[78] (**Figure 4**). The development of a novel viral strategy characterised in the publication, allowed for the number and position of each primary infected cell (starter cell) to be mapped, and control experiments revealed a cre-dependence and no unspecific labelling of the rabies virus (PAPER I, **Figure 1, Supplementary Figure 1 and 2**).

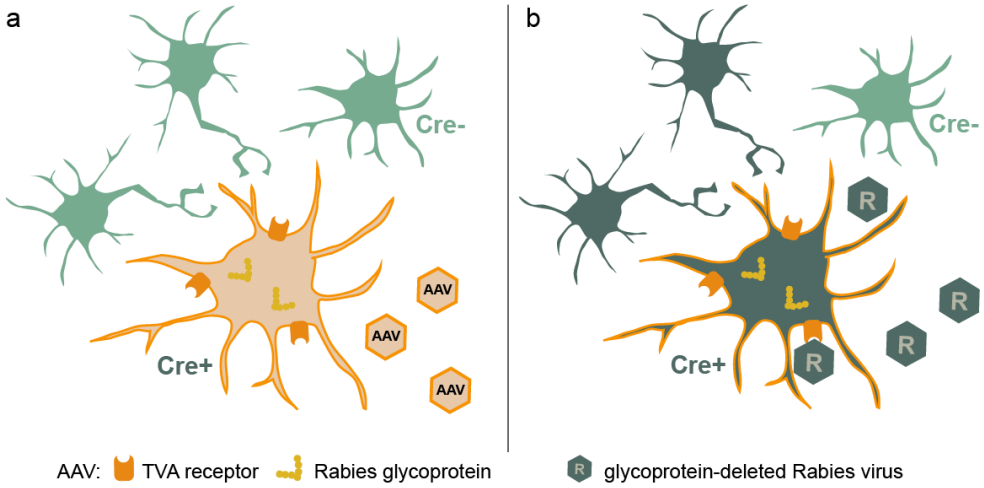

Figure 4. Novel Rabies virus tracing system[79]. (a) First injection, delivery of AAV with cre-dependent expression of the TVA receptor and Rabies glycoprotein. (b) Second injection, delivery of pseudo typed Rabies virus, only TVA expressing neurons take up rabies particles that will retrogradely jump one synapse due to the presence of rabies glycoprotein.

Although the rabies virus system developed displayed no unspecific labelling, there are other factors worth considering when discussing rabies virus labelling. The level of transsynaptic spread of a rabies virus system is indicated to be an underestimation of actual connectivity. In addition, the spread of the virus is dependent on the level of glycoprotein expressed by the starter cell, the number of rabies virus particles entering the starter cells, as well as the time available for transsynaptic spread[77]. Our novel rabies tracing system combined the expression of the TVA and rabies glycoprotein into one viral construct (PAPER I, **Figure 1, Supplementary Figure 1 and 2**). This construct thereby allowed for a controlled and thorough mapping of the starter cell population. Nonetheless, it has been reported that the downstream gene can show lower expression levels in a polycistronic construct[80], which would then result in a lower expression of the rabies glycoprotein than of the TVA receptor for our construct.

As a result, the transsynaptic spread could potentially be lower in our systems in comparison to those of others, where the TVA and rabies glycoprotein have been delivered by two separate viral constructs (PAPER I, **Supplementary Figure 1**)[81–83]. However, this was carefully considered in our experimental procedures, and the IRES constructs chosen (T2A) display the most equal expression rates of a bicistronic construct[80,84]. In addition, we allowed for two weeks of transsynaptic rabies spread before analysing the brains, almost twice the time than commonly used protocols[81–83], but within the timeline of avoiding rabies induced toxicity[85,86]. As a consequence, the methodology and experimental protocol developed for this project resulted in nearly 170 000 input neurons mapped from 462 discrete brain regions.

2.2.3 *Results and Significance*

One of many conclusions to draw from PAPER I (not already further discussed in the paper) was that the afferent connectivity of the PFC targets all neuronal types investigated equally. Except from the neuromodulatory input, there was little input to be found that preferentially would synapse on a specific cell type. Although previous work has traced the input to both GABAergic and excitatory neuronal types[82,87], the comparison of input to four distinct cell types in the cortex had not previously been done. Therefore, there was no previous documentation of how the input could differ between excitatory neurons and multiple interneuron types. The similarity discovered was therefore previously unknown, and can be portrayed as a valuable property of the cortex, since the computational power of a highly integrative area as the PFC would be lost, if only single neuronal types would be synapsed upon.

Two of the previously described subnetworks (*see A unique connectivity of the PFC*) could be identified in the connectivity; dmPFC and vmPC. These two subnetworks were identified in both the cortex (PAPER I, **Supplementary Figure 4**) and in the thalamus (PAPER I, **Figure 5 and Supplementary Figure 7**) and this was, importantly, independent of what neuronal type the monosynaptic input targeted. These findings were remarkable, since one could assume that both the efferent and afferent connectivity (i.e. excitatory neurons) would be necessary to identify the subnetworks of the PFC. Nevertheless, monosynaptic input to GABAergic interneurons likewise displays the connectivity patterns of the PFC subnetworks. Additionally, the interconnectivity of the PFC as a module previously discussed still holds true for all four neuronal types traced (PAPER I, **Figure 2**). The local input from the ipsi-PFC stands for the majority of all cortical input, for both the three interneuron types and for the excitatory population.

In PAPER I, we for the first time attempted to map the interconnectivity of interneurons in the PFC with monosynaptic rabies tracing. Traditionally done by electrophysiological whole-cell patch recordings in primary sensory areas[51,88], the local connectivity of interneurons within the PFC had not previously been investigated. The connectivity found was similar to previously documented data in primary sensory areas[51,88] although the postsynaptic partners of SST neurons could not be mapped (PAPER I, **Figure 3 and**

Supplementary Figure 6). The inability of the rabies virus to jump the synapse of an SST neuron was an important finding, and though we did not discover the mechanism behind this observation, it could be a possible aid in the future, for better understanding of rabies tracing limitations.

The main contribution to the field of systems neuroscience of this paper, is undoubtedly the website created, and the data distributed for any researcher to further investigate the connectivity of the PFC and other brain regions. The data is purposefully presented in several different units (proportion, raw neuron number, starter cell/ input neuron ratio), especially since rabies tracing by itself gives rise to a wide variation in cell counts and infection rate. Therefore, any comparison between animal and genotype is presented as proportion in the figures. It is, however, also important for fellow scientists to be provided with an approximate cell number, for planning future experiments with circuit specific targeting of genetic constructs such as opsins or activity indicators.

"There is an undisputed axiom: physiologically dissimilar elements have dissimilar structures. Reversing this statement, one may equally justifiably conclude that parts of organs that are structurally different must serve different purposes."

Korbinian Brodmann 1909

3 ON THE NEURONAL BASIS OF COGNITION, CHAPTER 3

3.1 Cognitive studies in the mouse

For any species, except humans, a language barrier prohibits our understanding of how the outside world is perceived by other species, and the best interpretation left is direct analysis of behaviour and action. Therefore, cognitive studies in the mouse are purely based on the behavioural readout, and more accurately, the ability of researchers to carefully observe, interpret and quantify behaviour. Any cognitive abilities ascribed to mice, or function attributed to the mouse PFC, is the result of researchers monitoring the behaviour of mice. Consequently, any property or function of the PFC described in this thesis is based on neuronal activity, or perturbation of activity, correlated to the behaviour observed and quantified, not necessarily all occurred behaviour.

3.1.1 *Neuronal correlates of cognition in the PFC*

Extensive work has been done in the mouse investigating the activity and necessity of the PFC during highly cognitive processes such as; working memory[89,90], rule switching [91], contextual discrimination[92–94] and decision making[95–98] (among many others). As a general rule, PFC activity can be correlated to goal directed behaviour[93,95–97,99–101] and an interference of PFC activity will disrupt the execution of goal directed behaviour[95,97,99,102]. Much due to the genetic targeting of neuronal subtypes and circuits in mice, the activity of distinct neuronal populations and pathways can be monitored or pertubated. This allows for an even deeper understanding of the neuronal computations taking place during cognitive tasks, and the direct contribution of cell types or neuronal populations defined by their projection site[90,95,98,100,101]. The expansion of methodology and genetic targeting have lead us nowhere closer to an absolute ‘state or computation of cognition’. Conversely, the neuronal activity patterns behind the cognitive processes mentioned appear quite diverse and the contribution of neuronal subtypes heterogeneous (from what we can understand today).

The focus of this thesis is on a cognitive process that can be perceived as a cornerstone of goal directed behaviour: attention. Attention, or engagement, is most likely a process also involved in the studies mentioned above, but investigated alone, the use of visual attentional tasks is a common way to pinpoint the neuronal underpinnings of attention in mice and primates. The presence of visual attention is evaluated by the ability of the animal to detect and report the location of a visual stimuli, either presented alone, or together with distractors[14,15,100,103–105].

3.2 Oscillations

Brain oscillations are systematic fluctuating electrical activity in the brain at a certain frequency. In mice, oscillations are most commonly recorded from an intracranial electrode recording the summated electrical activity of all extracellular currents of nearby neurons (referred to as local field potential, LFP). Intriguingly, the frequency of which the summated electrical activity fluctuates has been associated with different brain states, and will naturally occur during certain behaviours (sleep[106], locomotion[107,108], immobility[109,110], cognition[100,111] etc.). Importantly, single neuronal activity, registered as action potentials, is not necessarily synchronized to the LFP, neither are action potentials the source of the local oscillations.

However, a prominent theory of the purpose of oscillations is to create a temporal organisation of local or long-range network activity (i.e. action potentials). That is, by a fluctuating synchronous activity of a local network, i.e. a synchronous fluctuation of a depolarized or hyperpolarized state, a temporal window of opportunity will be created when input would have a higher chance to activate the region (elicit action potentials in the local excitatory neurons)[112]. Experimentally, this has been tested within the vibrissae system of mice. The detection of a discrete sensory stimuli was enhanced if the input reached the sensory cortex during the 'window of opportunity' of the oscillation cycle[113].

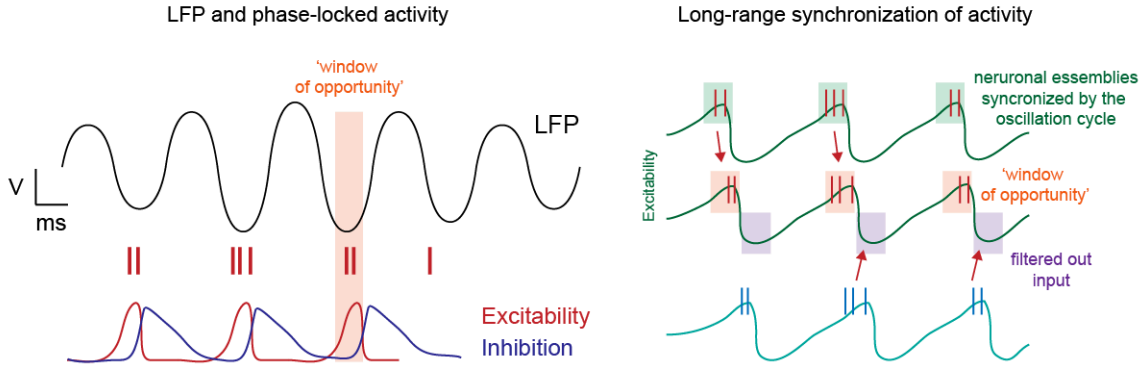

Figure 5. Theoretical framework of oscillations. LFP display a fluctuation in voltage dependent on the level of excitability or inhibition within the local network. Long-range synchronization of activity is more effective in transmitting information to downstream areas, desynchronised activity will be filtered out.

In the opposite direction, synchronous firing of multiple projection neurons would elicit a higher chance to activate, provoke an action potential, in a downstream target. Therefore, oscillations have been hypothesised to temporally synchronize assemblies of pyramidal neurons to optimize communication to a downstream region by synchronizing output[114]. With the development of 2-photon imaging of neuronal activity, neuronal assemblies (a subpopulation of excitatory neurons simultaneously or sequentially recruited during behaviour) have been identified in the PFC[60]. In addition, notably, single cell optogenetic reactivation of a neuronal assembly elicits the behaviour in which the activation of the

assembly was originally observed[115]. Consequently, a temporally precise reactivation of a neuronal assembly can provoke a downstream activation powerful enough to create a behavioural response. However, although experimentally perused[40], the direct evidence of oscillations creating or coordinating the activity of neuronal assemblies has not been experimentally examined. This is much likely due to experimental limitations, as a stable neuronal representation is currently most likely to be identified by 2-photon imaging, activity mapping on a timescale not possible to correlate to gamma oscillations.

3.2.1 *Gamma oscillations and visual attention*

A range of brain wave frequencies, i.e. between 30 Hz and 90Hz, are referred to as gamma (γ) oscillations. Two central properties of gamma oscillations make them relevant to study for cognitive neuroscientists. First, gamma bursts (gamma oscillations are not commonly continuous, but occur in bouts of activity) appear during cognitive states such as working memory[116] and attention[117]. Secondly, as mentioned earlier, one hypothesis regarding oscillations is the synchronization of the activity of a neuronal ensemble. The gamma cycle is approximately 10-30ms, a timescale that a down stream neuron would be able to integrate multiple input as one[118]. The presence of gamma oscillations during cognition could therefore be hypothesised to temporally organize the activity of pyramidal cells (to synchronously fire within the same cycle) to efficiently transmit and receive information[119].

Visual attention is a common framework to study gamma oscillations as it naturally occurs during attention, additionally, the visual system has a long history of study in the primate and the connectivity and flow of activity within the primate visual areas is thoroughly mapped[120]. Gamma oscillations have therefore been experimentally investigated in primates performing advanced visual tasks. Upon doing so, oscillatory synchrony within, and in-between visual areas, have been disclosed. At large, the presence and power of gamma oscillations is increased in both the PFC and visual cortex when the animal is allocating attention and correctly performing a behavioural task. Neurons in both the visual cortex and frontal areas that encode attended location or feature of the visual stimuli display enhanced phase-locking to the gamma cycle[19,121]. Additionally, long-range oscillatory synchrony has been found to occur during visual attention between neuronal groups encoding the same spatial location[15,122]. This has been hypothesized to enhance the communication between visual regions, since temporally precise activity would arrive to the downstream area when the 'window of opportunity' would be open (**Figure 5**)[123,124].

3.2.2 *Generating gamma oscillations*

As previously mentioned, oscillations are summated electrical activity, and not the readout of synchronous action potentials, since high frequency events, such as e.g. spikes, are commonly filtered out. The appearance of gamma oscillations is therefore the fluctuation of the activity of a large population of neurons, e.g. at a frequency of 40Hz. In the cortex, PV

neurons have been the long-time suspect in the generation of gamma oscillations as they are electrically coupled (allowing for fast internal synchronization) and elicit direct $GABA_A$ receptor mediated inhibition onto pyramidal cells. The time constant in which pyramidal cells could recover from $GABA_A$ –mediated inhibition (5-10ms) and depolarize (but not necessarily fire an action potential) fits with the frequency band of gamma oscillations[119,123]. Direct optogenetic activation of PV neurons in the cortex generates gamma oscillations in the somatosensory cortex of mice[125,126], but this has not been experimentally tested in other mammals (until now).

3.3 PAPER II

3.3.1 *Purpose and Aim*

The main purpose with this paper was to generate an additional animal model to study the relationship between, PV neuron activity, gamma oscillations and cognition. A wide variety of behavioural paradigms have been developed for the rat, often related to human tests used to diagnose psychiatric disorders. Although, several behavioural tasks designed for rats have been adapted to mice, a cognitive, social and behavioural difference between these two rodent species makes it relevant to study both[44,127].

The aim of PAPER II was to develop a transgenic rat with brain-wide and highly specific expression of Cre in PV neurons to allow for accurate and efficient expression of Cre-dependent viral constructs (opsins and biosensors). Subsequently, the developed transgenic rat strain was used to carefully characterize the in-vitro and in-vivo electrophysiological properties of PV neurons in the rat.

3.3.2 *Methodological considerations*

The main methodological consideration when creating and evaluating a new transgenic animal is the specificity and efficiency of the transcription of the inserted genetic construct. A thorough evaluation is a direct responsibility towards the neuroscientific community, and pivotal for the results and interpretations of any future study with the PV-cre rat. Common strategies that have been used to achieve cell type specific expression of viral constructs are cell type specific promoters, or the insertion of Cre into the rat genome by bacterial artificial chromosomes (BAC). Viral constructs are size-restricted and demand a ‘small’ cell type specific promoter to be used, this can make them hard to identify and often unspecific [128,129]. A BAC insertion of Cre into the rat genome relies on a random integration of the new sequence into the genome and requires the evaluation of several founder lines[130]. Additionally, there is little control over how many copies, or where within the genome the construct is integrated. This can, if not screened for carefully, create accidental knock-out rats. Therefore, we chose to take advantage of the Cas9/CRISPR system for a specific

integration of Cre into the genome[131](PAPER II, **Figure 1a and Supplementary Table 1**). After careful molecular characterization we found both reliable expression of Cre in PV neurons and effective recombination of Cre-dependent viral constructs (PAPER II, **Figure 1 and Supplementary Figure 1**).

3.3.3 *Results and Significance*

Following the confirmation of reliability of the novel PV-cre rat, the electrophysiological characterization of single and network activity of PV neurons are the main results of PAPER II. *In-vitro* characterization of the electrophysiological properties of PV neurons (as identified by the expression of cre-dependent ChR2-mCherry expression) displayed similar results as seen in PV neurons in mice[126] and in rat[132] (post-hoc identification) and again confirming the specificity of Cre expression (PAPER II, **Figure 2 and Supplementary Figure 2**). Careful consideration was put to map the distance of each patched cell to the surface of the brain in order to identify any electrophysiological changes due to the layering in which the cells abided by. The distribution of PV expressing basket cells and chandelier cells display a different distribution along the layering of cortex[88], and therefore it was of interest to see if any of their electrophysiological traits would be apparent in our data. So far, no significant changes were recognized between the PV neurons patched in different layers. Most likely a post-hoc analysis of the morphology of each neuron would be necessary to identify the presence of different subclasses of PV expressing interneurons. As chandelier cells are very sparsely distributed in the cortex, our sample size is possibly not adequate enough to differentiate the two subclasses.

The second part of PAPER II focuses on the single, as well as the network activity of PV neurons *in-vivo,* recorded by intracranial electrodes, either by chronically implanted tetrodes or acute silicone probes. PV neurons are identified by opto-tagging or post-hoc analysis of spike shape and amplitude[100,133]. Two highly relevant findings are described in the manuscript; first, light stimulation reliably activates channelrodopsin expressing PV neurons in the PFC, and as a consequence thereof, wide-spiking (WS) pyramidal cells where inhibited (PAPER II, **Figure 3**). This is of great importance to future studies as an indirect tool of local inhibition, where a powerful activation of PV neurons in a circuit can function as a transient lesion of a restricted brain area[134].

Secondly, a brief 40Hz light stimulation induced a prominent elevation of gamma oscillations in the LFP, and the activity (action potentials) of a single PV neuron displayed strong phase-locking to the gamma cycle. That is, the PV neurons kept firing within the same space in the cycle (the through) as previously described in mice[100]. Additionally, activating PV neurons in multiple frequencies (more specifically, activating PV neurons at 20-80Hz, the range of gamma oscillations) elevated the power of each frequency in the LFP. The amplification, i.e. power, was the highest at 35-45Hz, all indicating a similar circuit function of PV neurons in the rat as in mice[125,126] (PAPER II, **Figure 4 and 5**). Although both species investigated so far are rodents, the similarity in electrophysiological properties can indicate a conserved circuit function of PV neurons across different

mammalian species. The accessibility and possible manipulation of gamma oscillations in the transgenic PV-Cre rat strain opens up for a new range of experimental investigations. The relatively large size of a rat brain makes it more accessible for large-scale, multi-sight electrophysiological recordings (in comparison to mice), and in combination with this new access to manipulation of oscillation; intra area and long-range synchrony can be further investigated. The cognitive effect of either a forced synchrony or disruption of endogenous activity of multiple cortical areas could decisively confirm hypothesis established by primate multi-site recordings[123,124].

3.4 PAPER III

3.4.1 *Purpose and Aim*

Gamma oscillations have for long been associated with the allocation of visual attention[123], and more recently, experimentally generated by 30-80Hz synchronous activation of PV neurons[125]. The activity pattern of PV neurons, and their relationship to gamma oscillations, had however, never previously been shown during visual attention, and the necessity of their activation had not earlier proven vital for successful allocation of attention.

The aim of PAPER III was to identify and record the activity of PV neurons in the PFC during visual attention, as well as to investigate the cell-type specific activity in relationship to local network gamma oscillations. Additionally, we aimed to evaluate the necessity and/or requirement of frequency specific activation of PV neurons for successful allocation of visual attention.

3.4.2 *Methodological considerations*

A methodological concern of PAPER III is the use of the mouse as an experimental animal to study visual attention. Mice are not heavily reliant on their visual system and the behavioural task that was used was originally developed for rats (5-choice serial reaction time task)[33]. Although only 3 cues were used, and a longer training time was needed for mice in comparison to rats, all experimental animals reached a stable performance (PAPER III, **Supplementary Figure 1**). Notably, the occurrence of gamma oscillations during spatial visual attention had not previously been shown in mice (although it had been observed in primates). For this reason, the generation of gamma oscillations in the PFC during visual attention in mice was therefore not necessarily expected. It had however, previously been shown in rat, that discrete PFC lesions influenced behavioural performance in multiple aspects[135] and distinct electrophysiological patterns of activity in the PFC could be detected during a correct or incorrect response[34,41].

3.3.3 *Results and Significance*

The first result described in PAPER III was an increase in the firing rate of PV neurons during correct allocation of attention, and importantly, this activity could not be correlated to reaction time, nor was it dependent on the previous trial outcome (PAPER III, **Figure 2 and 3**). These findings indicated that the activity, and correlation observed were not encoding the execution of a motor program or a motivational state. Intriguingly the activity of WS pyramidal cells did not display a homologous activity pattern, but did instead separate into two groups that were either increasing or decreasing their mean firing rate during correct trials (i.e. correct allocation of attention). This is highly relevant with regard to the additional findings in the paper.

The increase in gamma power (i.e. increase in gamma oscillation amplitude) during correct trials (in comparison to error trials) was highly modulatory for the timing of singe cell action potentials. PV neurons displayed synchronous and phase-locked activity over all, with the strongest effect during correct trials. Significantly phase-locked PV neurons only fired an action potential during the trough of the gamma cycle (PAPER III, **Figure 4**). WS pyramidal cells however, only displayed significant phase-locked activity within the gamma cycle during successful allocation of attention. When investigated closer, significantly modulated WS neurons displayed two separate patterns of firing within the gamma cycle, i.e. either in the peak or the trough respectively. The two separate populations of WS neurons not only phase-locked to the gamma oscillations differently, but displayed different over-all firing patterns along the entire attentional period. WS neurons that showed phase-locked activity at the peak of the gamma oscillation displayed an overall decrease in firing rate during correct trials. In contrast, WS neurons firing in the trough of the gamma oscillation would increase their mean firing rate during a successful allocation of attention (PAPER III, **Figure 5**).

The importance of this finding lies in the timing of the PV neurons and the two WS neuronal populations' activity. As described earlier, the presence of LFP oscillations has been hypothesized to coordinate the timing of network activity. Although not yet experimentally proven, the results indicate that modulated PV neurons would synchronously follow (or give rise to) the local network activity, and therefore modulate the activity of WS pyramidal cells. WS cells eliciting an action potential simultaneously as the PV neurons would then have the 'opportunity' to increase their firing rate, while WS neurons firing in the peak of the gamma oscillation would be inhibited by the GABA release of PV neurons (i.e. increasing their firing rate, PAPER III, **Figure 5**).

The findings described in PAPER III, portray two different, or potentially simultaneous, effects of gamma oscillations in the PFC during visual attention. As described in the section *Oscillations,* local fluctuations of the LFP are suggested to indicate excitable or inhibited states within the local network, creating a 'window of opportunity' and synchronize local activity. Neuronal assemblies are therefore hypothesized to be coordinated by the presence of gamma oscillations to elicit a correct behavioural response. The WS

subpopulation displaying an increase of activity is potentially a neuronal assembly coordinated by local gamma oscillations. Additionally, the intriguing timing of the PV neurons and the subpopulations of WS neurons indicate that the activity of subpopulations of WS neurons could be promoted not only by local fluctuations in field potential, but in addition also by the recruitment of local GABAergic interneurons (i.e. PV neurons) that inhibit the activity on 'non-prioritized' neuronal assemblies.

However, as a synchronous activity of PV neurons can give rise to gamma oscillations, it is likely that the activity of PV neurons are the cause of both promoting the synchronous activity of a WS pyramidal assembly as well as simultaneously inhibiting the activity on non-promoted pyramidal cells. To solemnly ascribe the activity of a local cortical network to the frequency of the activity of PV neurons is probably incorrect, they are nonetheless a vital factor for the local network computations in the PFC during attention (PAPER III, **Figure 6 and 7**).

"*None of its cognitive functions can be understood if taken out of a broad connectionist context. Any hypothetical modularity of the PFC is functionally meaningless if taken out of wide-ranging networks that extend far beyond the confines of any given prefrontal area.*"

Joaquin Fuster 2001

4 CONCLUSIONS AND FUTURE DIRECTIONS

4.1 Conclusions

This thesis dissects the circuitry and network activity of distinct cell types in the PFC of the rodent. Originally portrayed in the human and primate, the PFC has more extensively been investigated in rodents by the use of modern neuroscientific techniques. As described in Chapter 2, the neuronal type can be considered a conserved unit throughout the evolution of amniotes brains. Although the composition and the distribution of neuronal types can differ between species, there is a potential evolutionary conserved functional aspect to neuronal types. Hypothetically, since today there is no clear evidence for this, neuronal types could display analogous functional properties within the brain circuitry of multiple species and could therefore be compared between rodents and humans. Consequently, the work in this thesis focuses on the circuitry and activity of genetically defined neurons (i.e. neuronal types).

A main focus of the work within this thesis is connectivity, and the similarity and diversity of the connectivity of neuronal types in the PFC. As cortical areas can seem similar in appearance, the connectivity of a cortical region is a highly defining factor in how to discriminate potentially unique functional units of the cortex. By carefully mapping monosynaptic input to four separate neuronal types, we show in PAPER I that all connectivity traits, defining the PFC, hold true for multiple neuronal types: the appearance of subnetworks within the PFC, the distinct thalamic innervation, and the high interconnectivity between PFC subregions. Additionally, although not included in the final version of PAPER I, a hierarchical index (SLN^{136}) could be calculated for each cortical input region to the PFC (**Figure 3**). Subregions of the PFC (ORB, AI) not containing starter cells, i.e. outside the injection site, ranked the highest and primary sensory regions (MOp, SSp) the lowest. More specifically, these findings indicated that fellow PFC regions display feedback-like projections to the rest of the PFC while primary sensory regions display classical feedforward projections to the PFC. Importantly, the projection patterns held for all four neuronal types targeted in the PFC, suggesting that both pyramidal cells and interneurons are subjected to an anatomically defined hierarchical organisation within the cortex. Further confirmed by long-range connectivity originating from discrete layers to an equal extent for each neuronal type targeted (Right panel, **Figure 3**). Possibly, these findings describe the importance of all investigated neuronal types in the PFC participating in the integration of brain-wide input, and that the activity of interneurons within a local circuit is not dominantly regulated by neighbouring neurons. Although fewer in numbers, the activity of interneurons, or more precisely the interplay between GABAergic interneurons and excitatory neurons, is regarded to be of great importance to the neuronal computations taking place in the cortex. One possible type of interaction, oscillations, is further described in Chapter 3.

The recordings and optogenetic manipulations of PV neurons, described in PAPER II and III, reveal specific interactions between excitatory cells and PV neurons in the cortex of the mouse and rat. The work carefully describes the timing and quantity of activity of PV neurons and WS pyramidal neurons, both during a state of attention, and throughout an artificial synchronous activity of PV neurons. PAPER II confirms previous findings in mice[125,126], where the synchronous activation of PV neurons gives rise to oscillations in the gamma range in the PFC and visual cortex of the rat.

In PAPER III, the innate occurrence of gamma oscillations during successful allocation of attention, the temporal synchrony of PV neurons' action potentials and the drop in behavioural performance during a perturbation of PV neuron activity, are all described. Together these data indicate a direct functional meaning to the occurrence of gamma oscillations and PV neuron activation in the PFC.

However, the topic of gamma oscillations often becomes circular, and similar to determining whether the egg (PV neurons) or the chicken (gamma oscillations) came first. Already in the vocabulary used, such as e.g. terms like phase-locking, indicating the timing within the gamma cycle, upon when the PV neurons fire action potentials. Now, if PV neurons were the single source of gamma oscillations, the logic would be reversed, and the cycle of the oscillation would be determined by the synchronous activity of PV neurons. The fluctuation in voltage recorded in the LFP, i.e. the oscillations, is suggested, for a large part, to be due to synaptic activity upon the dendrites of a local area[137]. Theoretically, the fact that PV neurons phase-lock in the trough of the gamma oscillation cycle (both naturally and optogenetically) suggests that the level of inhibition is the highest at the peak of gamma oscillations, and still this is where the voltage has increased, i.e. one finds a large amount of synaptic input. This could be due to pyramidal cell firing in the trough of the gamma cycle and a time delay of one or multiple synapses, that would then give rise to the elevation of the LFP. Likely, local LFP in a behaving animal is more complex than the above, and the oscillations observed are not solemnly due to the activity of PV neurons. PV neurons can however 'take advantage' of the situation to temporally prime a selection of pyramidal cells.

The occurrence of gamma oscillations in the cortex has led to a complex theoretical framework regarding the source of the voltage fluctuations observed in the LFP[138–140], and the exact relationship between inhibition, excitation and the actual LFP phase is still unclear. The temporal organisation of PV neurons, and the functional activity of excitatory neurons described in PAPER III, are at this stage, only observations, however, the activity of PV neurons was shown to be vital for the attentive state, and consequently, crucial for the network activity of the PFC.

PFC cell type specific activity and local circuitry, as well as their dependence upon each other, remains to be fully comprehended. The work described in this thesis, includes some steps towards solving the puzzle of the neuronal computations taking place in the PFC, by

carefully disentangling and observing small pieces of the computation at a time. Hopefully, in the future, we will be able to see the complete picture.

4.2 Clinical significance

The clinical significance of this thesis, focusing on the circuitry and activity of defined neuronal types, is of relevance to the pathophysiological network activity present in schizophrenic patients. On a cellular level, patients suffering from schizophrenia have repeatedly been shown to have a decreased amount of GABA synthesis present in the PFC PV neurons[141,142]. This is suggested give rise to abnormal oscillations in the cortex, and this could consequently potentially be a part of the pathophysiology seen in schizophrenic patients[143]. Although the cognitive impairments seen in patients suffering from schizophrenia are similar to the cognitive functions associated with oscillation, such as working-memory, perception and attention, causality is difficult to determine. However, studying the mechanism underlying the impaired functions of a diseased brain can be mutually beneficial for the understanding of cognition, and useful for designing future treatments of schizophrenic patients.

4.3 Future directions

Cognitive studies of the neuronal correlates of cognition in the mouse have seen remarkable improvements only during my years collecting data for this thesis. Behavioural paradigms have become more carefully designed, allowing for more careful observations of the behaviour of the animal. Although novel methodology has dramatically increased the quantity and complexity of data collected from a behaving animal, the neuroscience field is working hard in keeping up with appropriate hypothesis and analysis methods.

As for the PFC, correlating neuronal activity with behaviour is both dramatically easy and tremendously difficult. The complexity of neuronal activation, i.e. the high level of mixed selectivity, in the PFC makes it more or less possible to correlate neuronal activity with any (and many) behavioural readouts[144,145]. Additionally, searching for cognitive substrates in the brain is a complicated endower, and most commonly approached by trying to simplify the behavioural task, in order to avoid confounding factors, and in parallel extract the sought after computation; attention, decision, etc. Some of the ‘confounding factors’ can be sorted out by a highly repetitive tasks (collecting a lot of trials) such as neuronal activity, or “noise” encoding movement, salient sounds or other unwanted activity, are all greatly present on a trial-to-trial basis and can shape brain-wide activity[146]. The experiments performed in modern neuroscience are highly time consuming and have a high failure rate. Therefore, once a task and setup are up and running the aim and preferred tactic is generally to collect as much data as possible from the same behaviour. Hopefully,

methodological advancements and ambitious scientists will dare to combine multiple cognitive challenges to the same network. There is a potentially great benefit in comparing multiple states of cognition and the flexibility in-between to improve our understanding of the neuronal computations taking place during cognition.

The complex brain-wide connectivity scheme and neuronal composition of the PFC I hoped to have conveyed in this thesis, imply a highly integrative brain region. In addition, the integrated information assembled within the PFC is all encoded within a network projecting to a diverse population of brain regions at multiple processing levels. Cortex-wide imaging of neurons display a highly streamlined pattern of activity of cortical regions during behavioural tasks[95,99]. The coordination of this cortex-wide activity stem from the PFC, and consequently inhibiting the local network activity of the PFC disrupt both the behaviour and the cortex-wide coordination of activation[95,99]. Also relevant to the oscillatory activity observed in the PFC during cognitive states (Chapter 3), the mechanisms behind the communication between the PFC and downstream brain regions could be a key component to further decipher the neuronal computations enabling the PFC's 'functions'.

5 SUMMARY FOR NON-SCIENTISTS

Cognition is a common term for the mental process of acquiring knowledge of the world and using it. Cognitive problems, such as problems with working memory or concentration, are common symptoms of many psychiatric disorders such as: schizophrenia, attention deficit / hyperactivity disorder (ADHD), Parkinson's disease, bipolar disorder and obsessive-compulsive disorder (OCD). Cognitive impairment can be devastating to the patient's quality of life, and be a financial burden to society, and because of the lack of understanding of how a healthy brain calculates and solves cognitive challenges, there is little clinical help to treat cognitive symptoms.

The part of the brain named the prefrontal cortex (PFC) is indispensable for higher brain functions such as working memory, attention, and decision-making, and many mental disorders are suggested to occur due to an imbalance in the PFC. The PFC is not only the most advanced and developed brain region we have, but also the most sensitive one. In the PFC there are several types of neurons, some inhibitory, others excitatory. It has long been hypothesized that an imbalance in excitation and inhibition is one of the causes of mental illnesses, such as e.g. in schizophrenia and autism. The aim of the research in this thesis is to uncover the relationship between specific cell types, anatomical regions and the brain's higher cognitive abilities.

This thesis (PAPER I) describes a project in which we worked on mapping the connectivity of four unique nerve cells (both excitatory and inhibitory) in the PFC, to understand if this imbalance in the PFC is caused by signals from the rest of the brain. We also mapped the local connectivity to understand which of these nerve cells locally inhibit or excite each other in the PFC, to gain a deeper understanding of the local activity.

With a specially designed rabies virus, together with an advanced digital screening process, we mapped the exact position of all brain cells that send signals to the PFC in mice. This resulted in a published open-source atlas to facilitate researchers in our field with their future projects. Now that the connectivity to the PFC is established, several research labs, including our own, can begin to explore and dissect what kind of function each of these signals carries.

We have a particular interest in the cell types in the PFC that are suspected to be affected in patients suffering from schizophrenia (PV neurons) (PAPER II, PAPER III). To examine the function of these cells, we have used a relatively new technique, i.e. optogenetics, to turn these cells on and off in mice, that had been trained in demanding cognitive exercises. This approach can give us information with regard to a direct connection between a specific cell type, in a discrete brain region, with a certain type of cognitive capacity, such as attention, memory or decision-making.

A cornerstone of cognition is attention, as concentration must be distributed to relevant stimuli in order to perform a goal-directed, purposeful action. Reliably detecting and

processing information from the outside world, and simultaneously filtering out irrelevant information, is a critical process in the brain to solve cognitive challenges. Part of this thesis highlights how the brain prioritizes visual information from the outside world, which results in visual attention, to solve a cognitive task. More specifically, in PAPER III, we study how the PFC, the brain region responsible for cognitive control, can synchronize its activity to more reliably detect relevant visual stimuli.

We hope that the work presented in this thesis will in the future help us answer what kind of information reaches the PFC and how this information is processed so that higher cognitive processes can proceed in the brain. Hopefully, compiled data from our research will help us understand what can go wrong when someone gets sick.

Swedish;

Kognition är vanlig term för den mentala processen för att förvärva kunskap om världen och använda den. Kognitiva problem, så som problem med arbetsminnet eller koncentration, är vanliga symtom vid många psykiatriska sjukdomar som: schizofreni, uppmärksamhetsbrist/hyperaktivitetsstörning (ADHD), Parkinsons sjukdom, bipolär sjukdom och tvångssyndrom (OCD). Kognitiva funktionsnedsättningar kan vara förödande för patientens livskvalitet och en ekonomisk börda för samhället, och på grund av bristen på förståelse för hur en frisk hjärna beräknar och löser kognitiva utmaningar finns det lite klinisk hjälp för att behandla kognitiva symtom.

Den främre hjärnbarken (prefrontal cortex, PFC) är oumbärlig för högre hjärnfunktioner så som arbetsminne, uppmärksamhet och beslutsfattande, och många psykiska sjukdomar tros uppstå på grund av en obalans i främre hjärnbarken. Det är inte bara den mest avancerade och utvecklade hjärnregion vi har utan också den känsligaste. I den främre hjärnbarken finns flera typer av neuron, vissa inhibitoriska, andra excitatoriska. Det har länge hypotiserats om att en obalans i mellan excitation och inhibition är en av orsakerna bakom psykiska sjukdomar så som schizofreni och autism. Målet med forskningen i denna avhandling är att hitta att samband mellan specifika celltyper, anatomiska regioner och hjärnans högre kognitiva förmågor.

I denna avhandling (PAPER I) beskrivs ett projekt där vi jobbat med att kartlägga konnektiviteten av fyra unika nervceller (både excitatoriska och inhibitoriska) i främre hjärnbarken, för att förstå om denna obalans i främre hjärnbarken skapas av signaler från resten av hjärnan. Vi kartlagde även den lokala konnektiviteten, för att förstå vilka av dessa nervceller inhiberar eller exciterar varandra lokalt i den främre hjärnbarken för att få en djupare förståelse för den lokala aktiviteten.

I PAPER I har vi med ett specialdesignat rabiesvirus tillsammans med en avancerad digital screeningprocess mappat tillbaka den exakta positionen av alla hjärnceller som skickar signaler till främre hjärnbarken i möss. Detta resulterade i en publicerad och öppen atlas för att underlätta för forskare i vårt fält med sina framtida projekt. Nu när konnektiviteten till

främre hjärnbarken är fastlagt kan flera labb, inklusive vårt eget, börja utforska och dissekera vilken typ av funktion var och en av dessa signaler bär.

Vi har ett särskilt intresse för de celltyper i främre hjärnbalken (PV neurons) som misstänks vara påverkade i patienter som lider av schizofreni (PAPER II, PAPER III). För att utreda dessa cellers funktion, använder vi oss av en relativt ny teknik dvs. optogenetik för att slå av och på dessa celler i möss som tränats i krävande kognitiva övningar. Detta ger oss ett direkt samband mellan en celltyp, i en hjärnregion, med en viss typ av kognitiv kapacitet, så som uppmärksamhet, minne eller beslutsfattning.

En hörnsten i kognition är uppmärksamhet, eftersom koncentration måste distribueras till relevanta stimuli för att utföra en målstyrd, målmedveten handling. Att pålitligt upptäcka och bearbeta information från omvärlden och samtidigt filtrera bort irrelevant information är en kritisk process i hjärnan för att lösa kognitiva utmaningar. En del av denna avhandling (PAPER III) belyser hur hjärnan prioriterar visuell information från omvärlden, vilket resulterar i visuell uppmärksamhet, för att lösa en kognitiv uppgift. Mer specifikt, hur den främre hjärnbarken, hjärnregionen som är ansvarig för kognitiv kontroll, kan synkronisera sin aktivitet för att pålitligare upptäcka relevanta visuella stimuli.

Vi hoppas i framtiden att arbeten presenterade i denna avhandling, ska hjälpa att ge oss svar på vilken typ av information som når den främre hjärnbarken, hur denna bearbetas för att högre kognitiva processer kan fortlöpa i hjärnan, och att förstå vad som kan gå fel när någon blir sjuk.

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LIST OF ABBREVIATIONS

ACA	Anterior Cingulate Area
AI	Agranular Insular Cortex
ILA	Infralimbic Area
LFP	Local Field Potential
MOs	Secondary Motor Cortex
MOp	Primary Motor Cortex
ORBl	Lateral Orbital Cortex
ORBvl	Ventrolateral Orbital Cortex
PFC	Prefrontal Cortex
PL	Prelimbic Area
PV	Parvalbumin
SSp	Primary Somatosensory Cortex
SST	Somatostatin
SLN	Supragranular Layer Neurons
VIP	Vasointestinal Peptide
dm	Dorsomedial
vm	Ventromedial
vl	Ventrolateral

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